SHORT COMMUNICATION

Biological monitoring of exposure to *n*-heptane by gas chromatographic/mass spectrometric determination of its metabolites

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Urine samples from 10 workers that had been exposed to *n*-heptane were analysed by the GC/MS technique to verify the concentrations and the relative abundances of its metabolites. The procedure of sample preparation has undergone some modifications with respect to the Perbellini method and the mass spectrometric detection was carried out in selected ions monitoring conditions. The analyses of samples collected during three different workshifts showed that 2-heptanol was not the main metabolite and that the remains of 2-heptanone, valerolactone and 2,5-heptanedione were present at the beginning of the successive work-week at 12, 34 and 39% of the average values found at the end of the previous week. Overall, a very slow excretion rate was detected for the last metabolite. The main and significant metabolite at the end of the two workshifts was 2-heptanone which was detected in urine at average values of 413 and 238 μg g^{-1} creatinine. This urinary ketone correlated better than other metabolites with respect to the airborne *n*-heptane at the end of both the workshift and work-week. These preliminary data suggest that further studies should be carried out to confirm whether 2-heptanone is really useful as an *n*-heptane marker in biological monitoring.

Keywords: n-*heptane metabolites, glues, adhesives, biological monitoring*.

Introduction

In industrial processes the health risk is often linked to the use of low-boiling solvents such as aliphatic hydrocarbons. This occurs in shoe factories where the solvents contained in glues and adhesives are the dangerous compounds (Luedersorf *et al.* 1985, Marasi *et al.* 1986, Triebig and Schaller 1991, Streete *et al.* 1992, Valentini *et al.* 1994).

Recently a significant modification in the composition of the solvent mixture has been observed. The reason for these

variations is due to the introduction of some EEC regulations on the labelling of the toxic-harmful products, which has induced producers to change the composition of the solvent mixture. So, new products contain a lower concentration of *n*-hexane, but a higher content of *n*-heptane.

Low exposure to *n*-hexane leads to a low urinary excretion of 2,5-hexanedione with the consequent reduced risk to workers, while the increased presence of *n*-heptane makes the determination of its metabolites important. A sensitive and accurate analytical method is now essential to study the correlation between the *n*-heptane in the air and the urinary metabolites so as to single out a useful metabolite to biologically monitor the exposure. The scientific community has showed little interest in this solvent and its metabolites, as demonstrated by the few papers published (Frontali *et al.* 1981, Bahima *et al.* 1984, Perbellini *et al.* 1986, Valentini *et al.* 1994, Stormer and Fisher 1995). The pathway of its metabolic transformation has been proposed by Bahima *et al.* (1984) and Perbellini *et al.* (1986). In particular, the enzymatic oxidation of *n*-heptane leads to the formation of four isomeric heptanols, the 2- and 3-species being the most abundant. The 2-heptanol can be further hydroxylated to produce 2-heptanone or 2,6- and 2,5-heptanediol. Subsequently, these compounds can be oxidized to corresponding hydroxyketones which may be metabolized to 2,5- and 2,6-heptanedione. Moreover, the formation of γ-valerolactone probably proceeds by oxidation and deacetylation of 6-hydroxy-3-heptanone to yield 4-hydroxypentanoic acid which in turn lactonizes to the compound found.

Among all these metabolites, 2,5-heptanedione, like the corresponding compound in *n*-hexane, can have neurotoxic effects (O'Donoghue and Krasavage 1979).

This paper proposes a GC/MS method for the determination of the main metabolites, such as 2-heptanone (2-one), 2-heptanol (2-ol), 3-heptanol (3-ol), γ-valerolactone (lactone) and 2,5-heptanedione (dione) in the urine samples collected from the same workers during three different phases of the industrial cycle and makes some considerations about choosing the most suitable and bio-kinetically significant compound among the possible metabolites of the *n*-heptane absorbed. Moreover, the study emphasizes the trend in the release of target-compounds deriving from metabolized *n*-heptane.

MATERIALS AND METHODS

The study involved 10 workers exposed to *n*-heptane in a shoe factory in which the persons worked in a single 8-h workshift. Urine spot samples ($n = 30$) were collected from all the subjects within 1 h of the end of the first workshift (Monday), within 1 h of the end of last working day of the work-week and before starting the first shift of the successive work-week. The urine was frozen at -20°C prior to analysis. During each workshift concerned with urine collection, the workplace air was analysed so as to determine the respective exposure to *n*-heptane of each target worker. The personal sampling of airborne hydrocarbon was carried out by solid sorbent cartridge, containing charcoal coconut (100 mg/50 mg) at a flow of 0.6 l/min for 4 h. The analyses of the above cartridges were performed by the GC/FID system after desorption with 1 ml carbon disulphide, obtaining a percentage standard deviation of 6.3 on the overall method (Eller 1984).

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The five metabolites of *n*-heptane were analysed using a modification of the method of Perbellini *et al.* (1986). To a 5 ml urine volume derived from each of the samples collected was added 0.25 ml HCl (12 N), followed by heating at 100°C for 90 min in order to obtain the acid hydrolysis. After cooling, the hydrolysed sample was extracted with 5 ml dichloromethane. Subsequently, the organic phase was dried on sodium sulphate. To a 2.5 ml aliquot of the extract was added 4.4 µg exactly of *n*-butyl acetate (Bac), which was used as the internal standard, and following this the volume was reduced by nitrogen flow. One µl of solution was injected into the GC/MS instrument. Urine from non-exposed workers underwent the same hydrolysis and extraction treatments as urine from workers and was used as the reference.

All the metabolite measurements were performed using a Hewlett-Packard GC/MS system consisting of a 5890 II gas chromatograph and a 5971 A quadrupole. An HP Vectra 486 data system was used for the acquisition and elaboration of data.

The GC separation of analytes was obtained using an Ultra-1 (25 m, 0.2 mm i.d.) as the capillary column, under the following conditions: (a) for metabolites: column temperature, 55 °C for 3 min—5 °C min⁻¹—180 °C for 10 min; (b) for *n*-heptane: column temperature, 40 °C for 2 min—10 °C min⁻¹—100 °C for 5 min. For all the measurements the injector temperature was 250 °C, the interface/detector temperature, 280 °C and the splitless time, 0.2 min.

The mass spectrometric determination of the five *n*-heptane metabolites and internal standard was carried out by recording specific mass fragments (SIM) as reported in Table 1, obtained with an electron energy of 70 eV, a dwell time of 50 ms and a source temperature of 176 °C.

2-Heptanone, 2-heptanol, 3-heptanol, γ-valerolactone, *n*-butyl acetate, used as the internal standard, and HPLC grade dichloromethane were purchased from Aldrich, Milan, Italy. 2,5-Heptanedione was kindly supplied by L. Perbellini, Institute of Occupational Medicine of University of Verona, Italy. All the reference compounds had a purity of more than 98% as reported in the Aldrich catalogue and verified by GC/MS for dione.

In all the urine samples, the creatinine content was determined by the method of Jaffe, and metabolite values were expressed per gram of creatinine.

Results and discussion

The procedure for sample preparation for the GC/MS analysis, was subjected to some modifications, one of which consisted of the 5 ml urine used as the starting sample. Moreover, after acid hydrolysis, the aqueous solution was extracted with 5 ml CH_2Cl_2 and to a 2.5 ml aliquot of this phase (dried on sodium sulphate) was added *n*-butyl acetate, as an internal standard, and the volume subsequently reduced. The detection and successive determination of the metabolic species proposed by Perbellini *et al.* (1986), except 4-heptanone, was performed in selection ion monitoring, as reported in Table 1. Of the three

Metabolite	*a*	*b*	R^2
2-Heptanone	0.071	−0.25	0.994
3-Heptanol	0.045	+0.40	0.997
2-Heptanol	0.052	+2.32	0.997
Valerolactone	0.029	+2.38	0.997
Heptanedione	0.071	−0.77	0.999

Table 2. The regression coefficients, R^2, and constants *a* and *b* as calculated from the relationship $y = a x + b$ where y = peak area ratio of *n*-heptane metabolites vs internal standard and x = concentration of standard metabolite in µg l⁻¹.

analytes, 2-heptanone, 2- and 3-heptanol, only a single specific ion was monitored, while for lactone and heptanedione two characteristic ions were recorded. The last column shows the metabolite linearity ranges ($R^2 > 0.990$) useful for determinations in the real samples. The calibration curves were obtained with five different concentrations prepared using urine of unexposed persons spiked with suitable methanolic solutions of the investigated metabolites. Peak areas for standards and internal standard were measured from GC/MS-SIM traces and the ratio plotted against concentration of each metabolite. Regression coefficients, R^2, and constants *a* and *b* were calculated from the relationship $y = a x + b$ where y = area ratio and x = metabolite concentration in µg l⁻¹. Such values for all the *n*-heptane metabolites studied are reported in Table 2.

The detection limits were 6, 10, 8, 20 and 10 µg l⁻¹ for 2-one, 2-ol, 3-ol, lactone and dione, respectively, with a signal-to-noise ratio of 3. The relative standard deviation (coefficient of variation) for four replicate determinations of spiked urines containing 77, 61, 100, 528 and 80 µg l⁻¹ of 2-one, 3-ol, 2-ol, lactone and dione, respectively, was less than 8%. The recovery method was evaluated as being about 91%. In particular, two 5 ml urine samples spiked with an average concentration of the investigated metabolites, were submitted to the same extraction, drying and volume reduction treatments as the real samples. The respective GC/MS data were compared with those obtained from the methanolic standard solution (containing the same metabolite amounts) directly analysed without any preliminary treatment.

Figure 1 shows a typical chromatogram of a urine extract. The instrumental sensitivity for all the metabolites allowed the assignment of significant values for 96% of the metabolites in samples collected at the end of the shift and work-week against 72% of the useful detections at the beginning of the week. The most abundant metabolites in the Monday samples were 2-heptanone, valerolactone and 2,5-heptanedione.

The average values of each metabolite during the respective workshifts are reported in Table 3 where the real increase in the metabolite concentrations at the end of shift and work-week is shown. At the beginning of the first workshift of the following week the remaining average values of 2-heptanone, valerolactone and 2,5-heptanedione were 12, 34 and 39% respectively of the average amounts detected at the end of previous work-week.

In the investigations reported in the literature (Bahima *et al.* 1984, Perbellini *et al.* 1986), the main part among the *n*-heptane metabolites is assigned to 2-heptanol while

Metabolite	MW	rt (min)	Ions	Standard (µg l⁻¹)
2-Heptanone	114	7.52	58	20–512
3-Heptanol	116	7.98	59	30–1020
2-Heptanol	116	8.10	45	25–2000
Valerolactone	100	8.81	56, 85	105–10560
Heptanedione	128	11.40	99, 57	30–1152
Butyl acetate	116	5.42	43	I.S.

Table 1. Molecular weight, retention time, selected ions and concentration of standard solutions of *n*-heptane metabolites and internal standard.

	Concentration (μg g^{-1} creatinine)				
	2-Heptanone	3-Heptanol	2-Heptanol	Valerolactone	Heptanedione
A	413 (70–826)	243 (48–650)	188 (nd–1063)	4431 (1234–13552)	197 (77–400)
B	238 (96–525)	241 (29–778)	237 (nd–1048)	3547 (1368–9614)	167 (93–447)
C	29 (9–84)	8 (nd–26)	5 (nd–11)	1209 (178–1397)	65 (31–114)

Table 3. Average concentrations and range (μg g^{-1} creatinine) of five *n*-heptane metabolites determined in urine of 10 exposed persons during three different workshift phases: at the end of first working day of work-week (A), at the end of the last day of the same work-week (B) and before the starting of the first workshift of the successive week (C).

Key: nd, not detected.

heptanedione is sometimes undetectable. In these experimental determinations the 2,5-heptanedione species were always found in the urine samples, but 2-heptanone was considered the predominant metabolite, after the lactone was excluded due to its low specificity. The measured concentrations of *n*-heptane in air were in the range of 15.3–91.7 mg m^{-3}, corresponding to an average value of 46.1 mg m^{-3}. The regression coefficients, R^2, and constants a and b, reported in Table 4, show a possible correlation between the solvent values in the air and the biological monitoring data. In particular, using the urines from the end of the workshift, *n*-heptane in the air significantly correlated with 3-heptanol, 2-heptanone and 2,5-heptanedione, while for the samples at the end of the work-week the solvent value correlated with 2-heptanone, only. 2-Heptanone was clearly the metabolite with the highest R^2 value in both situations (Valentini *et al.* 1994). Regarding the 2-heptanol, no correlation has been drawn from data of the airborne C$_7$ hydrocarbon and this metabolite in urine. Furthermore, a comparison of the urinary concentrations of the metabolites studied during the different workshifts demonstrated that the excretion of metabolized *n*-heptane extended over time leading to the varied release of metabolites. Looking at the metabolic pathway, the 2- and 3-heptanol species are generated in the first metabolic step, while 2-heptanone, but in particular, valerolactone and 2,5-heptanedione appear in the ultimate phases of the biotransformation process and only after a longer time (Bahima *et al.* 1984). The high residual level of heptanedione at the beginning of the first shift of the successive work-week was surprising, as if the persistence of this metabolite in the excreted urines were due to the continuous exposure to heptane. Similar behaviour was also reported recently by Stormer and Filser (1995) where the exposure to *n*-heptane in the 100–500 ppm range led, after 72 h, to the highest and constant level of 2,5-heptanedione in urines. This trend is typical of a kinetically slow metabolic process. Therefore, from
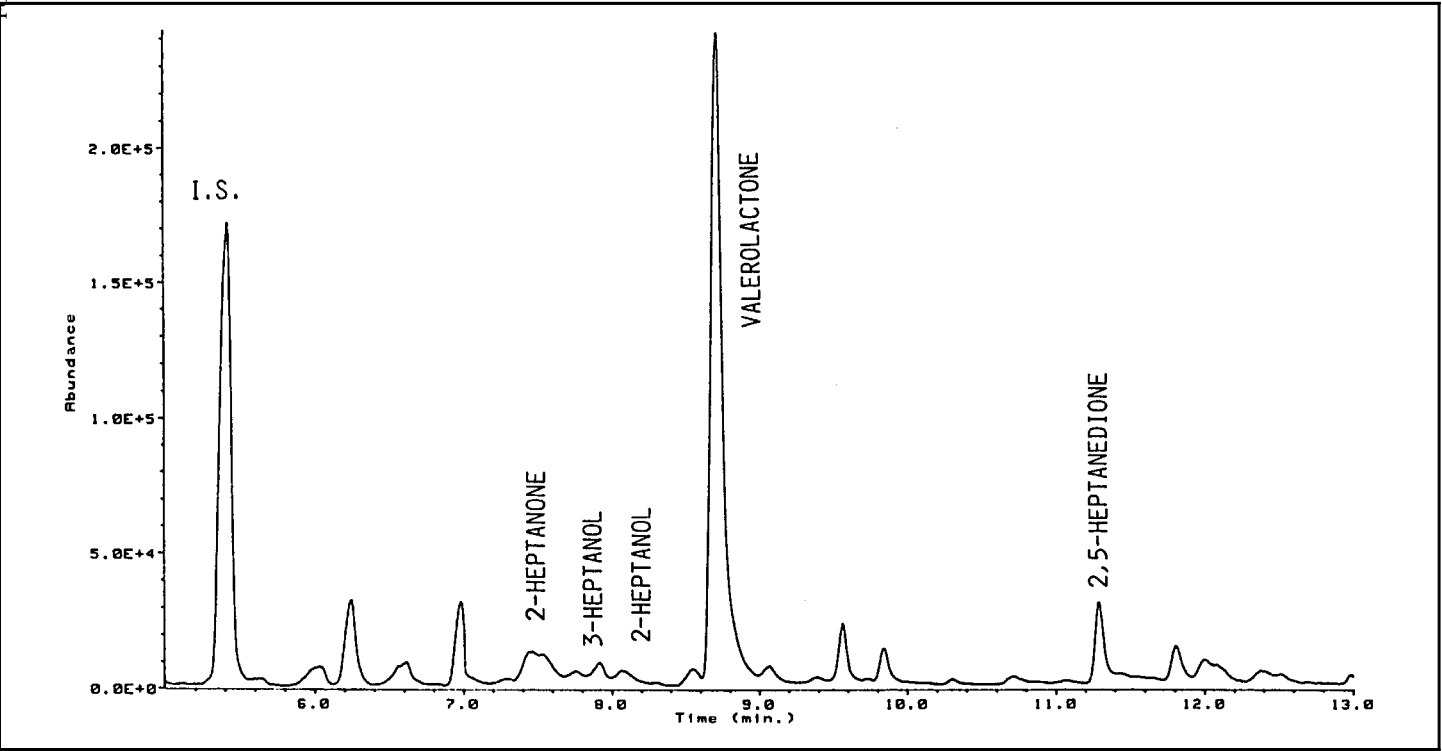
Figure 1. Typical GC/MS-SIM chromatogram of *n*-heptane metabolites detected in urine extract operating under the following instrumental conditions: GC (25 m×0.2 mm i.d. Ultra-1 capillary column submitted to 55 °C for 3 min—5 °C min^{-1}—180 °C for 10 min and injection in splitless for 0.2 min); MS (EI, 70 eV with acquisition in single ion monitoring of specific ions for each target metabolite).

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